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Granulomatous Encephalitis in Whipple's Disease

Electron Microscopic Observations

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Summary. Among the 21 autopsied cases of Whipple's disease on file at the Armed Forces Institute of Pathology, 4 had neurological symptoms and prominent involvement of the central nervous system, By light microscopy the lesions were composed of nodular aggregates of macrophages having cytoplasm that stained a distinctive pale blue with hematoxylin-eosin and bright red with the periodic acid-Schiff technique. By electron microscopy the cerebral lesions revealed bacilli morphologically identical to and undergoing the same sequence of degenerative changes as those observed in the intestine. The periodic acid-Schiff reaction stains the walls and the capsular material that persists even after intact organisms disappear. These observations further support the infectious nature of Whipple's disease.

Zusammenfassung. Von 21 Fällen von Whipplescher Krankheit in der Sammlung des Armed Forces Institute of Pathology zeigten 4 Fälle neurologische Symptome mit besonders ausgeprägten Hirnläsionen. Lichtmikroskopisch bestanden die Herde aus knötchenförmigen Anhäufungen von Makrophagen, deren Cytoplasma mit Hämatoxylin-Eosin eine charakteristische schwachblaue und nach Durchführung der Perjodsäure-Schiff-Reaktion eine stark rote Färbung zeigte. Elektronenmikroskopisch fanden sich in den Hirnläsionen Bacillen, die morphologisch den im Darm beschriebenen glichen und die auch dieselben Degenerationserscheinungen aufwiesen. Die Perjodsäure-Schiff-Reaktion färbt Kapselmaterial und Zellwände, die auch nach Untergang der Bacillen noch nachweisbar sind. Diese Beobachtungen werden als weiterer Beweis für die infektiöse Ätiologie der Whippleschen Krankheit angeführt.

Key-Words: Whipple's Disease — Granulomatous Encephalitis — Electron Microscopy.

In 1907 Whipple reported a new condition characterized by accumulations of foamy macrophages and lipid deposits in the submucosa of the small intestine and in mesenteric lymph nodes. He termed this condition intestinal lipodystrophy, focusing attention on the prominence of abnormal lipid deposits. Although he was uncertain about the etiology, he did report the presence of rod-shaped organisms in the mesenteric lymph nodes. Subsequently, many more reports were published, establishing that the disease could be diagnosed by the presence of periodic acid-Schiff-positive glycoprotein particles in the foamy macrophages (Black-Schaffer, 1949) and that the condition involves many organs (Upton, 1952; Farnan, 1958; and Sieracki, 1958), including the brain (Sieracki *et al.*, 1960; Krücke and Stochdorph, 1962; Lampert *et al.*, 1962; Badenoch *et al.*, 1963; and Smith *et al.*, 1965).

Haubrich *et al.* (1960) were among the first to study Whipple's disease by electron microscopy. They observed mononuclear cells which they interpreted as being mutant reticular cells that elaborated the PAS-positive material. Cohen

et al. (1960) described macrophages containing sinuous membranous sacs and extracellular dense bodies that they considered to be microorganisms. Yardley and Hendrix (1961) recognized these bodies as bacteria. Chears and Ashworth (1961) concluded that the undigested capsular material of the bacteria gave rise to the typical PAS-positive particles in the macrophages.

The demonstration that a favorable clinical response could be achieved with antibiotic therapy (England *et al.*, 1960; Davis *et al.*, 1963; Trier and Phelps, 1963) provided further evidence that bacteria play a role in the pathogenesis of Whipple's disease. Trier *et al.* (1965) made the critical observations that the bacteria are absent from the intestine of antibiotic-treated patients during remission but reappear with clinical relapse.

While there are now numerous electron microscopic studies of the intestinal lesions in Whipple's disease (Kurtz *et al.*, 1962; Kent *et al.*, 1963; Trier *et al.* 1965; Dobbin and Ruffin, 1967; Phillips and Finlay, 1967), we are unaware of any such studies of the cerebral lesions. In this report we wish to draw further attention to the encephalitis that may accompany or even precede the other manifestations of Whipple's disease and to demonstrate that the cerebral lesions contain bacilli and their derivatives identical to those described in the intestine.

Material and Methods

In the files of the Armed Forces Institute of Pathology, which include case material from military, Veterans Administration, and civilian sources, there are 21 autopsied cases of Whipple's disease. Fifteen had been previously reported by Enzinger and Helwig (1963). Among the additional cases four showed prominent cerebral involvement and were available for this electron microscopic study. A histochemical study of one of these cases has been reported previously (Lampert, 1962). The patients were all Caucasian males whose ages ranged from 44 to 53 at the time of death. All four patients had displayed marked disturbances of mental function. Brain tissue was available from all four cases for light and electron microscopic study. Small intestine was present in one case and mesenteric lymph nodes in two of the cases.

For light microscopy, paraffin-embedded sections were stained with hematoxylin-eosin, periodic acid-Schiff, Gomori methenamine-silver, and Gram stains.

For electron microscopy, the formalin-fixed tissue was post-fixed in 1% phosphate-buffered osmium tetroxide, dehydrated, and embedded in Epon. Sections 2 microns thick were stained with paraphenylenediamine, and suitable blocks were selected for further cutting. The thin sections were stained with uranyl acetate and lead citrate and were examined with a Siemens IA electron microscope.

Results

Light Microscopic Findings

Viscera. The small intestine showed accumulations of macrophages in the mucosa and submucosa. The individual villi were widened by the cellular infiltrate and contained cystic spaces, but the overlying epithelium was unremarkable. The macrophages in the mucosa and the submucosa were rounded and possessed amphophilic foamy cytoplasm. These cells were stained intensely by the periodic acid-Schiff (Fig. 1 a) and the Gomori methenamine silver techniques. In addition, there were scattered lymphocytes, plasma cells, and polymorphonuclear leukocytes.

Mesenteric lymph nodes showed multiple irregular cystic spaces that obscured the usual follicular pattern. These were occasionally lined by multinucleated giant cells. Admixed with the lymphocytes were large foamy macrophages. As in the

intestine, these stained strongly with the periodic acid-Schiff (Fig. 1 b) and Gomori methenamine-silver techniques. Interstitial and capsular fibrosis were prominent.

Other visceral organs were unremarkable except for isolated cells that stained by the periodic acid-Schiff technique.

Brain. The brains from all four cases showed similar lesions consisting of nodular aggregates of macrophages measuring up to 0.2 cm in diameter. The component cells had a rather distinctive appearance, inasmuch as the cytoplasm stained pale blue with the hematoxylin-eosin (Fig. 2 a). It was stained intensely by the periodic acid-Schiff (Fig. 2 b) and Gomori methenamine-silver techniques.

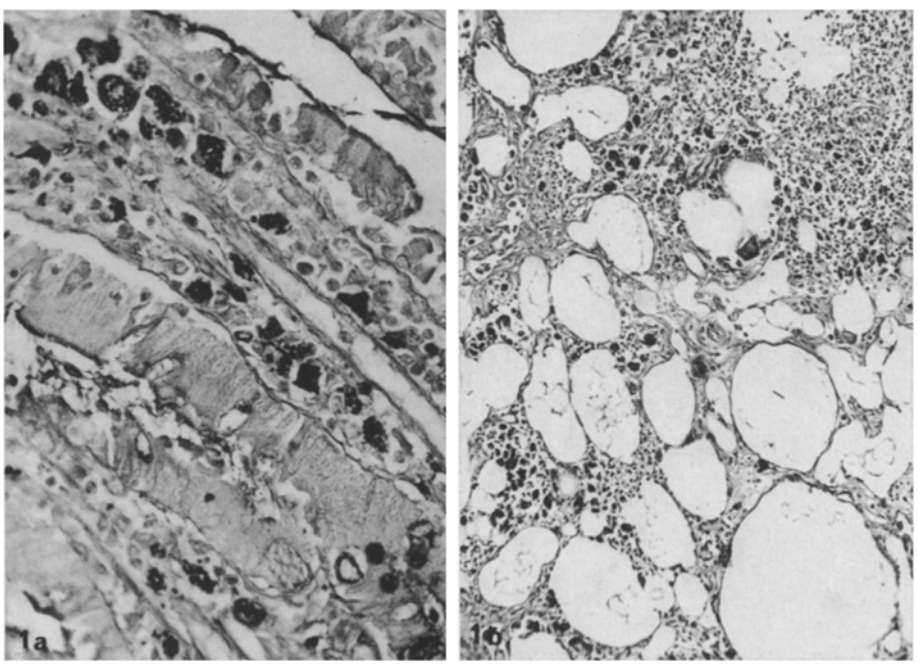

Fig. 1. a Small intestine from a case of Whipple's disease with cerebral involvement, showing periodic acid-Schiff staining of the macrophages in the mucosa. × 300. AFIP Neg. No. 68-1667. b Mesenteric lymph node from a case of Whipple's disease with cerebral involvement, showing irregular spaces and PAS-staining macrophages. × 80. AFIP Neg. No. 68-1666

There were rare small mononuclear cells about the nodular aggregates, although perivascular infiltrates of lymphocytes and plasma cells were prominent in one case. Occasional perivascular macrophages were also present in this particular case. These granulomas were randomly scattered throughout the cortical and subcortical gray matter of the cerebrum, the nuclear gray matter of the brain stem, and the cortical and nuclear gray matter of the cerebellum. Lesions were also diffusely distributed throughout the white matter, but they were less numerous and the individual cells were not so closely aggregated. Some nodules of the foamy, faintly basophilic cells were in a subependymal location (Fig. 3 a). These elevated

and/or protruded through the overlying ependymal cells and were associated with a proliferation of the subependymal astrocytes. These lesions had the same staining characteristics as the other cerebral granulomas (Fig. 3b).

Electron Microscopic Findings

By electron microscopy, a broader spectrum of cerebral lesions could be demonstrated. In the one case with a prominent perivascular inflammatory infiltrate, there were occasional large macrophages containing innumerable electron-dense fusiform structures (Fig. 4). These appeared as rods, ovals, or circles, depending upon the plane of section, but had maximal dimensions of 0.2×2.0 microns. The

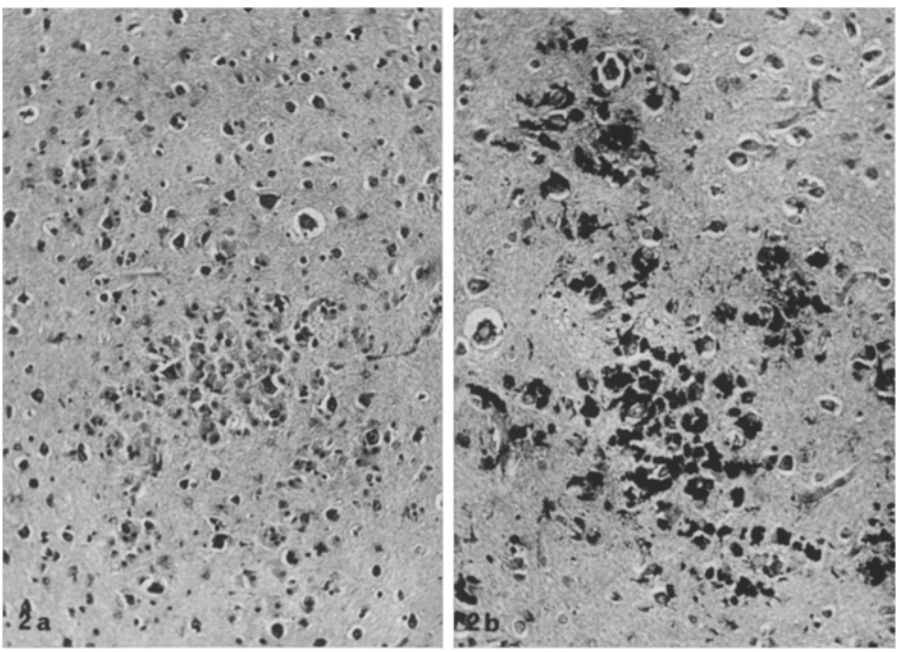

Fig. 2a and b. Cerebral cortex from a case of Whipple's disease, showing granulomas composed of macrophages. a The cytoplasm stains a distinctive pale blue with hematoxylin-eosin. $\times$ 115. AFIP Neg. No. 68-1668. b The cytoplasm stains intensely with the periodic acid-Schiff technique. $\times$ 145. AFIP Neg. No. 68-1670

protoplasm of the organisms was surrounded by a thin, electron-dense cell wall, which was in turn surrounded by a smooth, homogeneous, moderately, electron-dense capsule (Fig. 5a). Within these same macrophages were small aggregates of moderately dense membranous arrays (Fig. 5b).

More frequently, however, the macrophages contained organisms that appeared to be in various stages of degeneration (Figs. 6a and 6b). These were characterized by uneven clumping of the protoplasm and large, irregular, unbounded lucent areas. Occasionally fibrillar material corresponding to bacterial nucleoids could be recognized. Where the protoplasm was less dense, the bilaminar structure of the cell wall was apparent. As the degenerative changes progressed, the previously

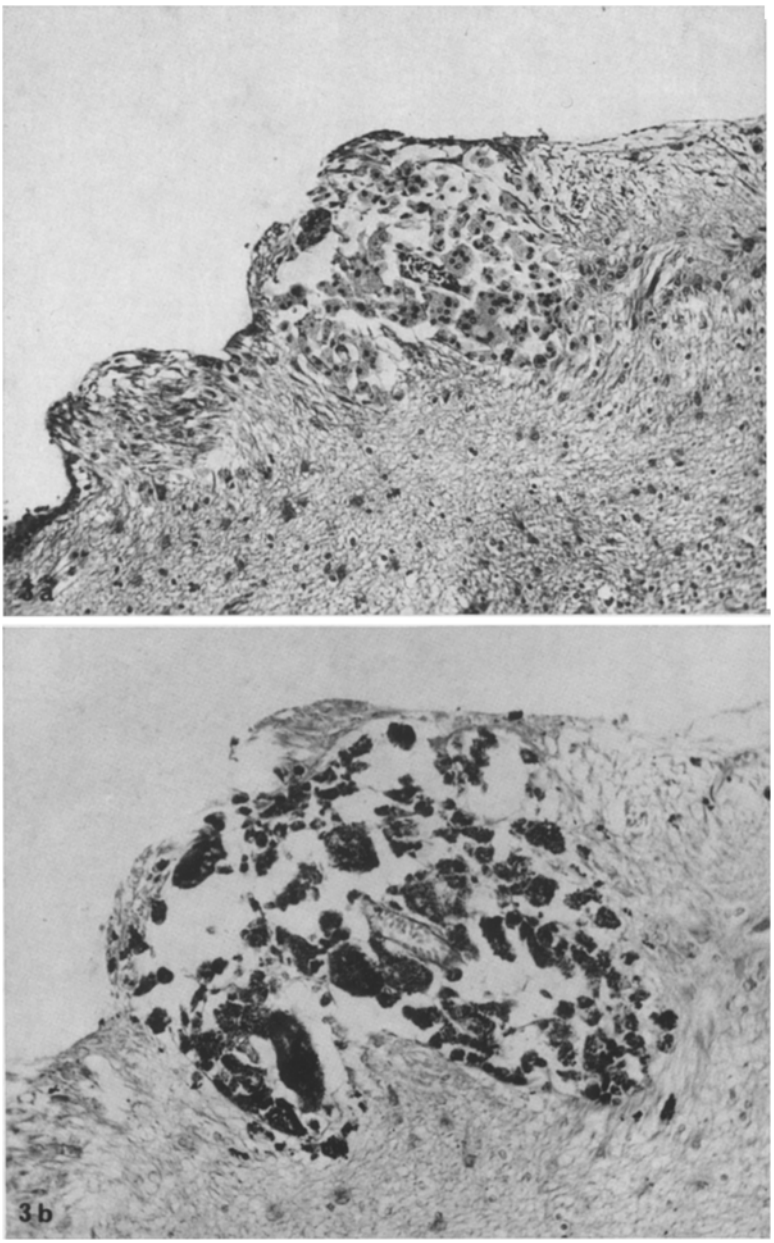

Fig. 3a and b. Subependymal granulomas in a case of Whipple's disease with cerebral involvement. a Note the pale blue foamy cytoplasm. H.-E. ×115. AFIP Neg. No. 68-1673. b These macrophages also stain intensely with the periodic acid-Schiff technique. ×180. AFIP Neg. No. 1675

smooth capsule became serrated (Figs. 6a and 6b). Evidence of binary fission (Fig. 6c) was observed and indicated that these degenerated organisms were once proliferating.

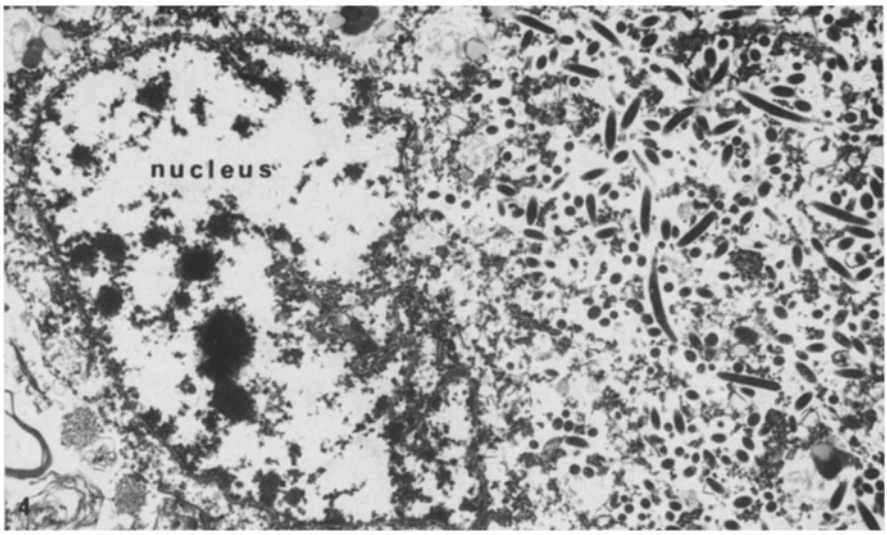

Fig. 4. Macrophage in a cerebral granuloma from a case of Whipple's disease. Note the abundant organisms in the cytoplasm. Uranyl acetate-lead citrate. ×4,000. AFIP Neg. No. 68-3257-7

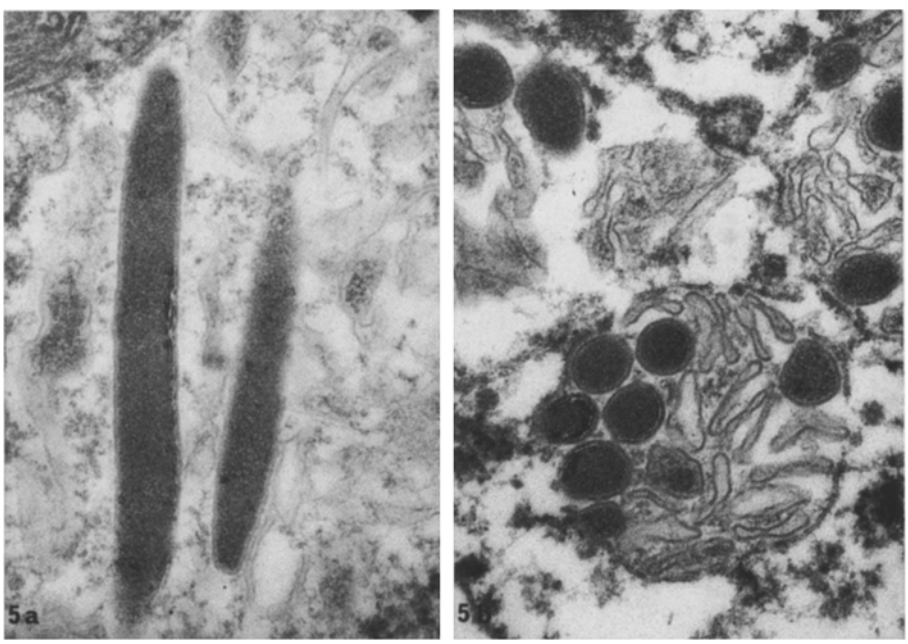

Fig. 5a and b. Intact organisms from a cerebral granuloma. a Longitudinal section showing the homogeneous osmophilic protoplasm and smooth capsule. Uranyl acetate-lead citrate. ×50,000. AFIP Neg. No. 68-3251-6. b Transverse section showing the organisms associated with a few serpiginous membranes. Uranyl acetate-lead citrate. ×50,000. AFIP Neg. No. 68-3251-5

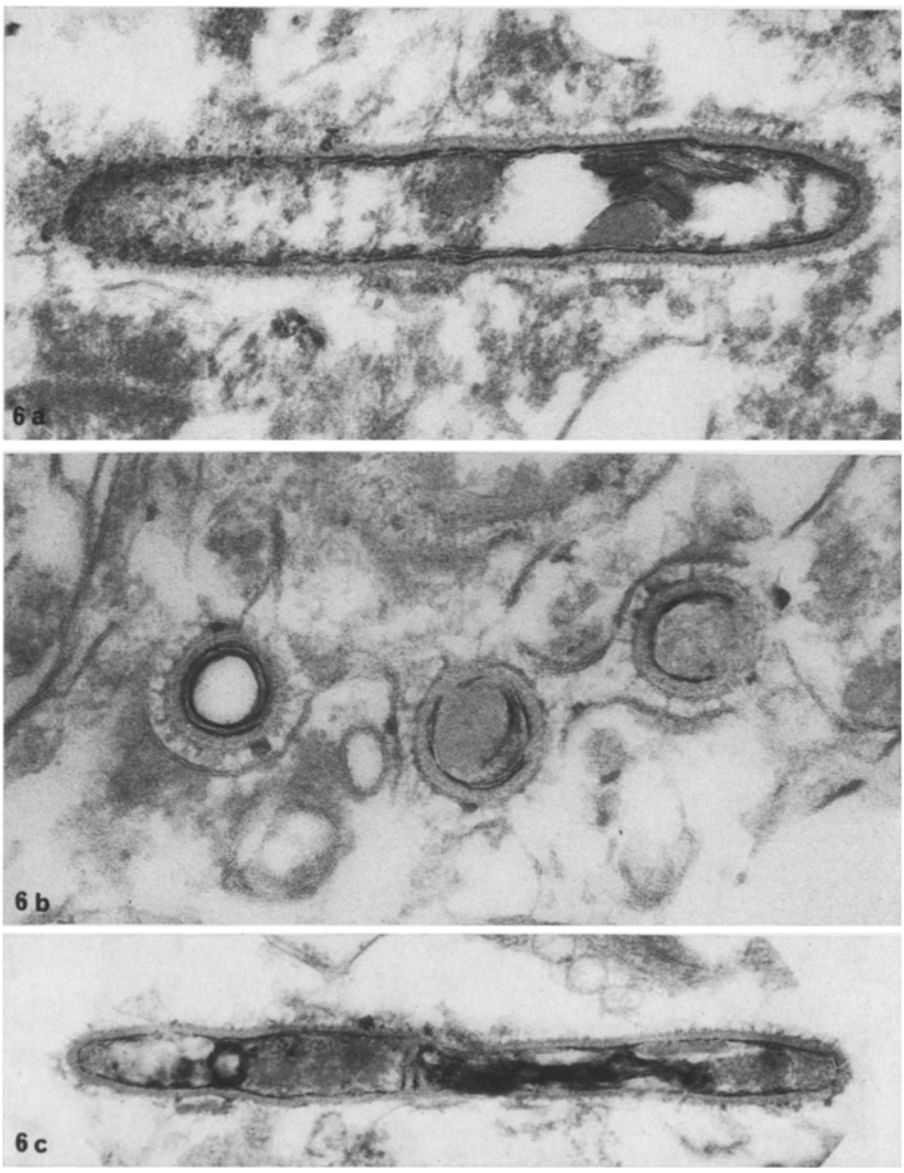

Fig. 6a—c. Degenerating organisms from the granulomas in a case of Whipple's disease with cerebral involvement. a Longitudinal section showing clumping of the protoplasm, unbounded lucent areas, and serration of the capsule. Uranyl acetate-lead citrate. ×75,000. AFIP Neg. No. 68-3251-4. b Transverse section showing serration of the capsule. Uranyl acetate-lead citrate. ×75,000. AFIP Neg. No. 68-3251-3. c Longitudinal section showing evidence of binary fission. Uranyl acetate-lead citrate. ×60,000. AFIP Neg. No. 68-3251-2

As the degeneration advanced, the organisms became less numerous and the accumulations of collapsed serpiginous membranes became more abundant. These often formed irregular intracellular masses 5 to 15 microns across that occasionally

indented the nucleus. Many of the macrophages contained only accumulations of membranes or only a rare recognizable organism (Fig. 7), and in two of the cases they were the only type of macrophages observed in the cerebral lesion.

The subependymal aggregates displayed the same sequence of changes within the macrophages as did the parenchymal granulomas. The associated astrocytes showed abundant glial filaments characteristic of proliferated fibrillary astrocytes.

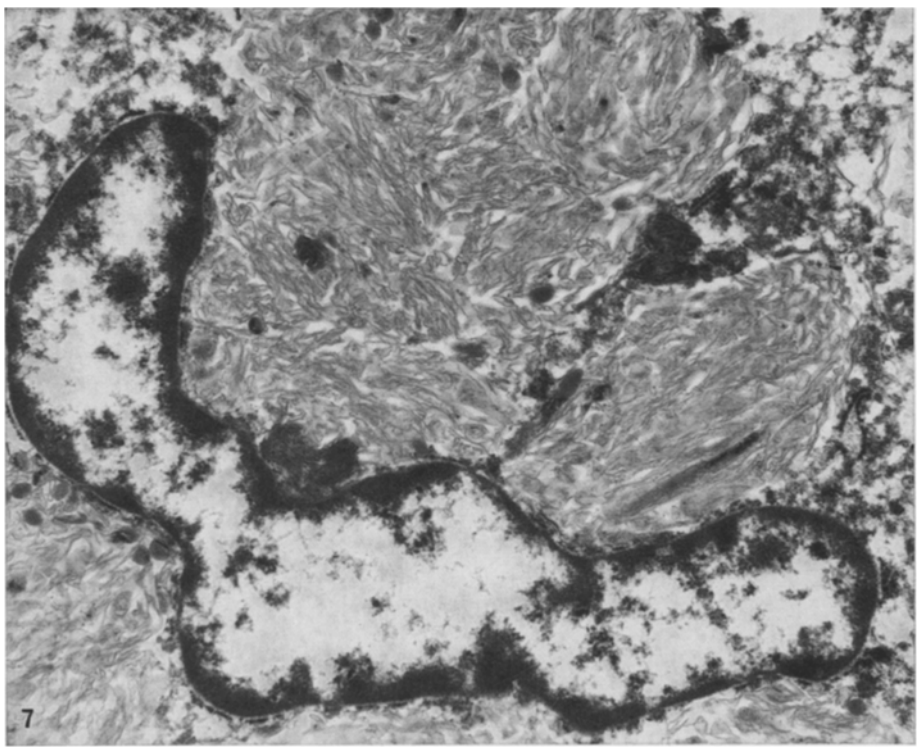

Fig. 7. Macrophage from a cerebral granuloma containing masses of serpiginous membranes and two degenerating organisms. Uranyl acetate-lead citrate. ×12,000. AFIP Neg. No. 68-3251-1

Discussion

Mental and neurologic symptoms have been described in a number of cases of Whipple's disease, although this is not commonly regarded as a feature of this condition. Enzinger and Helwig (1963) mention mental disturbances, psychoses, or disorientation in one of their cases and in six from the literature. Krücke and Stochdorph (1962), Badenoch *et al.* (1963), and Smith *et al.* (1965) each report single cases with mental symptoms and histologically demonstrable cerebral involvement. These cases are comparable to ours, in which all four demonstrated symptoms of a presenile dementia. On the other hand, not all cases with cerebral involvement display such symptoms (Sieracki *et al.*, 1960; Krücke and Stochdorph, 1962; Enzinger and Helwig, 1963; and Smith *et al.*, 1965).

Light microscopic study of all four of our cases revealed similar cerebral lesions. Most often these consisted of nodular aggregates of macrophages in the gray matter. Involvement of the white matter was less frequent, and the granulomas were less compact. There were also subependymal accumulations of the macrophages that elevated or ruptured the overlying ependyma. In all locations the macrophages displayed a distinctive pale blue staining with hematoxylin-eosin and deep staining by the periodic acid-Schiff and Gomori methanamine-silver techniques. Inflammatory cells were rarely encountered about the granulomas, although a perivascular infiltrate of lymphocytes and plasma cells was prominent in one case. Astrocytic proliferation was prominent around the granulomatous lesions.

By electron microscopy greater variation could be demonstrated in the lesion among the four cases than was apparent by the light microscopy.

In one case macrophages were found containing innumerable bacilli, each surrounded by a smooth capsule. More often the macrophages contained an admixture of degenerating organisms and collapsed serpiginous membranes. As the bacilli degenerated, the protoplasm became irregularly clumped and the capsule became serrated. In two of the four cases, the macrophages contained predominantly large masses of collapsed membranes and only a rare recognizable organism.

Thus it appears that the cerebral involvement in Whipple's disease is due to the presence of organisms that are morphologically identical to and undergo the same sequence of degeneration as the bacilli found in the intestinal lesions (Trier *et al.*, 1965; Dobbins and Ruffin, 1967). Our observations would support the concept that the membranes are remnants of bacterial walls and capsular material (Kurtz *et al.*, 1962).

Since bacterial cell walls are heteropolymeric compounds containing carbohydrate moieties (Perkins, 1963), it is understandable that the periodic acid-Schiff and the Gomori methenamine-silver techniques would not distinguish between apparently viable organisms and residual masses of membranes. This accounts for the uniformity of staining among our four cases, in two of which the electron microscopic study demonstrated varying numbers of organisms with a preponderance of membranes. Similarly explained are the observations that intestinal biopsy specimens from both untreated and antibiotic-treated cases of Whipple's disease display periodic acid-Schiff staining whereas the organisms themselves can be demonstrated only before treatment or during clinical relapses (Trier *et al.*, 1965; Dobbins and Ruffin, 1967; Kurtz *et al.*, 1962). Furthermore, this reconciles the present concepts with the occasional electron microscopic reports in which no organisms were identified (Adams *et al.*, 1963; Fisher, 1962; and Hollenberg, 1962) and with the older concepts that related Whipple's disease to a disorder of glycoprotein metabolism (Haubrich *et al.*, 1960; Fisher, 1962) or absorption (Black-Schaffer, 1949; Upton, 1952).

Attempts to isolate the organisms in culture from the intestinal biopsy specimens have met with limited success. Caroli *et al* (1963) and Kent *et al.* (1963) have isolated species of *Corynebacterium*, while Kok *et al.* (1964) and Kjaerheim *et al.* (1966) favor a *Hemophilus* species. Sherris *et al.* (1965) were unable to isolate any organisms and considered those previously reported as contaminants. Never-

theless, it seems most likely that the bacilli visualized in the intestine are of etiologic significance in Whipple's disease. Organisms of this nature have not been seen in specimens from controls (Trier *et al.*, 1965; Phillips and Finlay, 1967), the presence of the organisms parallels the clinical activity of the disease (Trier *et al.*, 1965; Dobbins and Ruffin, 1967; Kurtz *et al.*, 1962), and a variety of organisms would be expected if they were merely secondary invaders (Trier *et al.*, 1965). We feel that our demonstration of morphologically identical bacilli in the brains of patients with Whipple's disease further supports this assumption.

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